

artefact which is in the form of a deletion of one nucleotide at the Eam1105I site, transforming this site into a unique PshAI site. The resistance marker of the plasmid pRPA-BCAT99 was then changed by cloning, between the AatII and PshAI sites, an approximately 1.07 kb AatII-PshAI fragment prepared after PCR amplification of the gene encoding chloramphenicol resistance from the matrix pACYC184 (New England Biolabs #401-M), using the primers cm1 and Cm2, the sequence of which is:

Cm1 : 5'-CCCCCCGACAGCTGTCTTGCTTCGAATTCGTCC (SEQ ID NO: 4)

Cm2 : 5'-TTGACGTCAGTAGCTAACAGGGAGGG (SEQ ID NO: 5)

The plasmid thus obtained was called pRPA-BAT123. It was then modified by eliminating the *trpR* gene in the form of an approximately 0.525 kb SacI-Bst1107I fragment, and reclosing the plasmid after forming blunt ends with the Pfu polymerase (15 minutes at 75 C in the buffer recommended by the manufacturer Stratagène, and in the presence of 0.2 mM of deoxynucleotides). The plasmid thus obtained is the plasmid pRPA-BCAT127, the map of which is represented schematically in Figure 2.--

In the Sequence Listing:

Delete the Sequence Listing and insert the Sequence Listing submitted herewith.

**REMARKS**

The Notification of a Defective Response indicated that the Sequence listing filed May 30, 2001 was not in compliance with 37 CFR 1.821-1.825.

Enclosed is a substitute paper copy of the Sequence Listing, a computer disk containing the Sequence Listing in computer readable form and a Statement to Support Filing And

Submission In Accordance with 37 CFR 1.821-1.825. The Sequence Listing has been amended to comply with 37 CFR 1.821-1.825. The specification has also been amended to insert references to the sequence identifiers.

An early and favorable Office Action is requested.

Respectfully submitted,

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Date: October 9, 2001

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### MARKED UP REPLACEMENT PARAGRAPHS

**Replacement paragraph for the paragraph running from page 18, line 19 through page 19, line 25**

The 1.27 kb fragment [counting] containing the  $P_{trp}$  promoter, the ribosome binding site of the  $\lambda$  phage cII gene (RBScII) and the nitrilase gene of *Alcaligenes faecalis* ATCC8750 (*nitB*) was extracted from the plasmid pRPA6BCAT6 (application FR 96/13077) using the EcoRI and XbaI restriction enzymes, so as to be cloned into the vector pXL642 (described in CIP application No. 08/194,588) opened with the same restriction enzymes. The resulting plasmid, pRPA-BCAT15, was opened with the StuI and BsmI enzymes, and the 4.3 kb fragment was ligated with the purified 136 bp StuI-BsmI fragment of pRPA-BCAT4 (application FR 96/13077) so as to produce the plasmid pRPA-BCAT19. The partial sequencing of pRPA-BCAT19 confirmed the replacement of the codon of the Asp279 residue of the nitrilase with the codon of an Asn279 residue. The 1.2 kb EcoRI-XbaI fragment of pRPA-BCAT19 containing the  $P_{trp}$ : :RBScII: :*nitB* fusion was then cloned into the vector pRPA-BCAT28 opened with the same enzymes, so as to produce the 6.2 kb plasmid pRPA-BCAT29. The vector pRPA-BCAT28 was obtained by ligating the 3.9 kb SspI-ScalI fragment of pXL642 (CIP application No. 08/194,588) with the 2.1 kb SmaI fragment of pHG45ΩTc (Fellay *et al.*, 1987, Gene 52: 147-154) in order to replace the ampicillin resistance marker with the tetracycline resistance marker. In destroying the NdeI site close to the origin of replication of the plasmid pRPA-BCAT29 by partial NdeI digestion and the action of *E. coli* Polymerase I (Klenow Fragment), a plasmid pRPA-BCAT41 was obtained, the map of which is represented in Figure 1. The sequence of the expression cassette is represented by sequence identifier No. 2 [(SEQ ID NO 2)] (SEQ ID NO: 2).

*Alcaligenes faecalis* nitrilase is represented by SEQ ID NO: 3.

**Replacement paragraph for the paragraph running from page 21, line 9 through page 22 line 9**

The polyamide hydrolase gene of *Comamonas acidovorans* N12 described in application WO 97/04083 (*pamII*) was cloned into the vector pBCAT41. This polyamide hydrolase gene was amplified by PCR in the form of a 1.26 kb DNA fragment, while introducing, in the PCR primers, the EcoRI and NcoI restriction sites in the 5' position of the gene and the XbaI restriction site in the 3' position. This fragment was then treated successively with the EcoRI enzyme and Mung Bean nuclease. After extraction of the proteins with phenol-chloroform-isoamyl alcohol, the treatment was continued with an XbaI digestion. Similarly, the vector pRPA-BCAT41 was opened with the NdeI enzyme, and then treated with Mung Bean nuclease. After extraction of the proteins with phenol-chloroform-isoamyl alcohol, the treatment was continued with an XbaI digestion. After ligation of these two samples, the plasmid pRPA-BCAT43 was obtained: it contains the *P<sub>trp</sub>* promoter and the RBScII binding site separated from the translation start codon of the *pamII* gene by the sequence:

AATACTTACACC (SEQ ID NO: 6).

**Replacement paragraph for the paragraph running from page 40 line 7 through page 41 line 9**

After elimination of the unique NdeI site of the plasmid pRPA-BCAT30 by digestion and formation of blunt ends with polymerase I (Klenow fragment), the *trpR* gene was extracted from this latter plasmid in the form of an approximately 300 bp fragment prepared by treatment with the AatII enzyme followed by the action of polymerase I (Klenow fragment), and then, after inactivation of the reaction mixture, by digestion with the SacII enzyme. This fragment was cloned into the pRPA-BCAT66 plasmid after opening this plasmid with TthIII followed by

treatment with polymerase I (Klenow fragment) and, after inactivation, with SacII. The plasmid pRPA-BCAT82 was thus obtained. Its origin of replication was replaced with that of the plasmid pRPA-BCAT41-531 by replacing the approximately 1.12 kb Bst1107I-Eam1105I fragment. The construct selected during this cloning, the plasmid pRPA-BCAT99, has an artefact which is in the form of a deletion of one nucleotide at the Eam1105I site, transforming this site into a unique PshAI site. The resistance marker of the plasmid pRPA-BCAT99 was then changed by cloning, between the AatII and PshAI sites, an approximately 1.07 kb AatII-PshAI fragment prepared after PCR amplification of the gene encoding chloramphenicol resistance from the matrix pACYC184 (New England Biolabs #401-M), using the primers cm1 and Cm2, the sequence of which is:

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The plasmid thus obtained was called pRPA-BAT123. It was then modified by eliminating the *trpR* gene in the form of an approximately 0.525 kb SacI-Bst1107I fragment, and reclosing the plasmid after forming blunt ends with the Pfu polymerase (15 minutes at 75 C in the buffer recommended by the manufacturer Stratagène, and in the presence of 0.2 mM of deoxynucleotides). The plasmid thus obtained is the plasmid pRPA-BCAT127, the map of which is represented schematically in Figure 2.